The seed of life – investigating *Epichloë* embryo colonisation

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INTRODUCTION

Asexual cool-season grass endophytes of the genus *Epichloë* are strictly vertically disseminated via host seed. During the plant's sexual reproductive cycle, these fungi colonise the floret through the base of the ovary and subsequently develop in the nucellus tissue that surrounds the embryo sac. At seed maturity, the endophyte is distributed primarily in the embryo and in-between the aleurone and pericarp layers[1]. However, there is conflicting evidence on the timing of embryo colonisation.

AIM

Determine when *Epichloë* endophyte hyphae enter the host grass embryo.

MATERIALS & METHODS

- Biological materials: Tall fescue (*Festuca arundinacea*) cultivar 'Jackal' infected with *Epichloë coenophiala* strain AR601.
- The distribution of endophyte hyphae was microscopically tracked at five distinct plant developmental stages (Figure 1):
  - Early embryo sac stage (Stage I)
  - Mature embryo sac stage (Stage II)
  - Early fertilised stage (Stage III)
  - Young seed stage (Stage IV)
  - Mature seed stage (Stage V)
- Endophyte hyphae were labelled with fluorescent dyes and observed with confocal microscopy.

Figure 1A–E. Micrographs of ovaries or seeds from Stage I to Stage V. Bars = 500 µm.

RESULTS & DISCUSSION

Endophyte hyphae were observed in all stages of seed development from Stage I to Stage V. The *Epichloë* hyphae were easily observed in all flower and seed tissues including the early stages before fertilisation (Figure 2A). At Stage II, the ovary length had increased and the embryo sac vacuole enlarged where hyphae were observed attached to the large antipodal cells (Figure 2B). Later at Stage III, hyphae could be seen in-between the young embryo cells at the base of the ovary near the micropyle (Figure 2C).

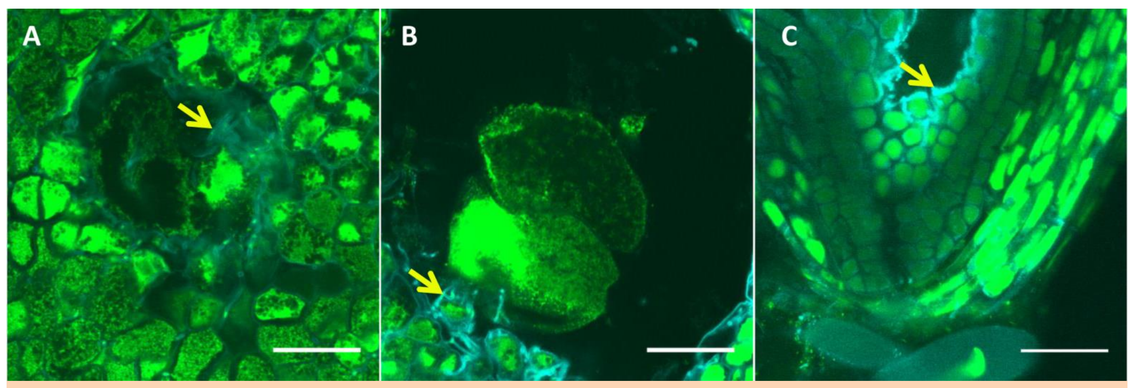

Figure 2 A–C. *Epichloë* colonisation within the grass embryo sac (see arrows) from Stage I to Stage III. Bars = 50 µm.

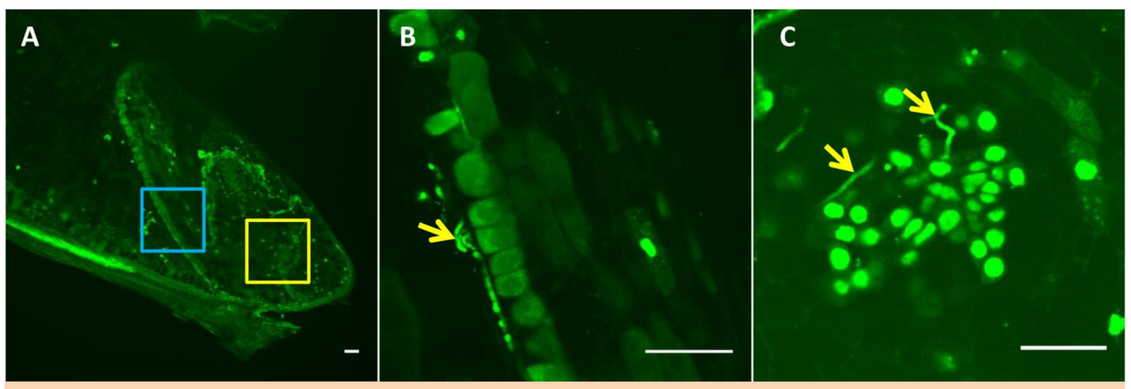

Figure 4A–C. Distribution of *Epichloë* hyphae in mature seed (A). (B, C) Magnification of the area within the blue and yellow boxes in A showing the endophyte hyphae in the infection layer and embryo axis, respectively. Bars = 50 µm.

With the onset of seed filling, the nucellus layer was crushed by the developing endosperm (es) (Figure 3A, B, C). Cross sections of ovaries at Stage III and Stage IV (Figure 1) showed that endophyte hyphae were found among the pericarp cells, the vascular bundles outside the ovule (vb), the nuclear projection (np), and the outer and inner integuments (oi, ii) (Figure 3E, F). From Stage II to Stage IV, more endosperm started to accumulate inside the embryo sac where endophyte was observed in-between the non-starchy endosperm cells (Figure 3F).

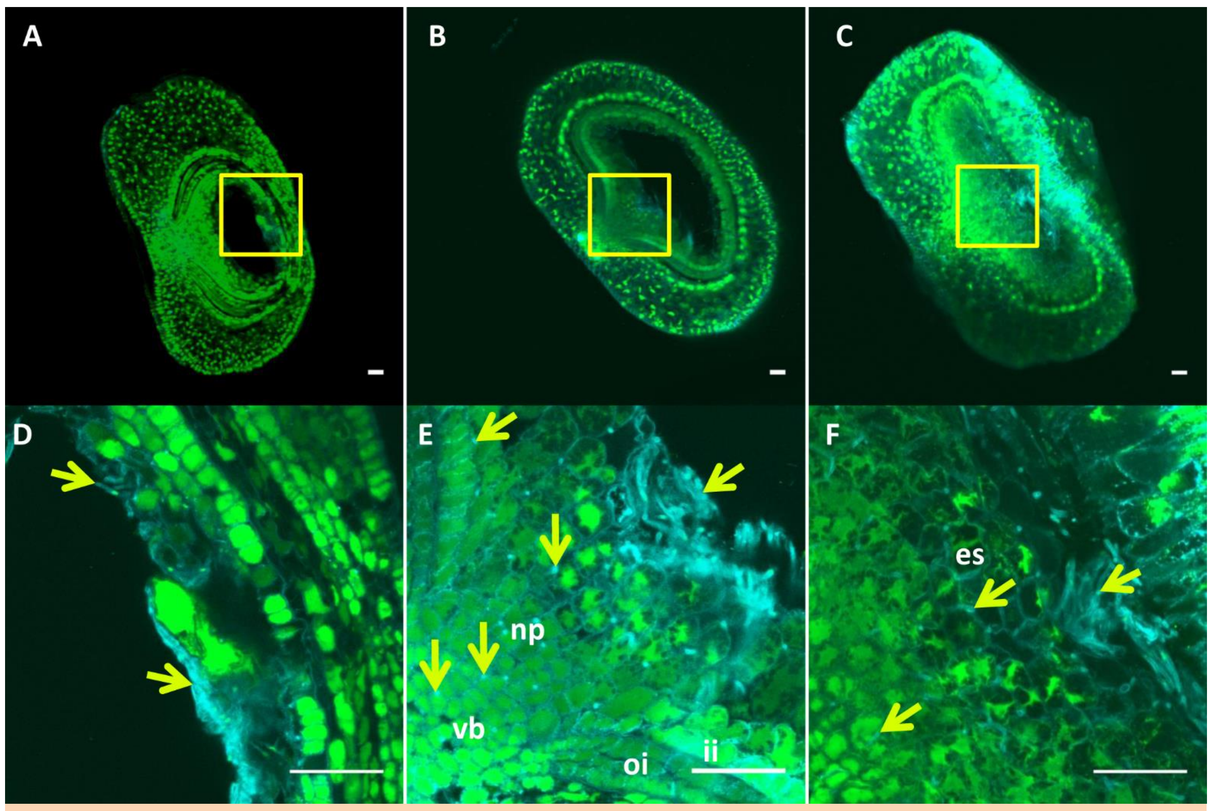

Figure 3A–F. Cross sections of plant reproductive structures showing the distribution of *Epichloë* hyphae at Stage II (A, D), Stage III (B, E) and Stage IV (C, F). (D, E, F) Magnification of the area within the yellow box in A, B and C, respectively. es, endosperm; ii, inner integuments; np, nuclear projection; oi, outer integuments; vb, vascular bundles. Bars = 50 µm.

Fluorescent staining indicated that viable endophyte hyphae were present in the 'infection layer' and embryo axis in mature seed (Figure 4A, B, C).

CONCLUSION

Host seed colonisation is an essential part of the *Epichloë* lifecycle and very few researchers have investigated this area in detail. We show, using fluorescent confocal microscopy, that endophyte hyphae enter the flower tissues at a very early stage and are associated with the embryo sac before fertilisation takes place, and later the embryo axis in the mature seed.

REFERENCES

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